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Memory Effects of Water Deprivation in European Beech (*Fagus sylvatica* L.) and Silver Fir (*Abies alba* Mill.) Seedlings Grown in Mixed Cultivation

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Citation: Yang, F.; Du, B.; Burzlaff, T.; Dutta, S.; Dannenmann, M.; Quan, X.; Maurer, D.; Rennenberg, H. Memory Effects of Water Deprivation in European Beech (*Fagus sylvatica* L.) and Silver Fir (*Abies alba* Mill.) Seedlings Grown in Mixed Cultivation. *Forests* **2022**, *13*, 1704. <https://doi.org/10.3390/f13101704>

Academic Editor: Roberto Tognetti

Received: 8 September 2022

Accepted: 12 October 2022

Published: 16 October 2022

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Abstract: Very limited information is available on the drought tolerance of European beech and silver fir in mixed cultivation, both for mature forests and natural regeneration. Particularly, little information is available regarding the significance on memory effects of drought exposure. Therefore, drought memory was analyzed in seedlings of these species grown in mixed cultivation in the present study. The results showed that previous-year drought hardening mediated enhanced biomass accumulation of beech leaves and root in the subsequent year, but did not impact fir growth. Total carbon (C) content was decreased by drought hardening in both the leaves and roots of beech and previous-year needles and roots of fir, in beech probably as a consequence of increased growth. Previous-year drought hardening had no significant effect on relative water contents, total nitrogen (N), or soluble protein contents in leaves and roots of beech and fir, but resulted in decreased total amino acid contents of beech leaves and fir needles. It further reduced structural N in current-year fir needles and decreased C/N ratios in roots of both beech and silver fir seedlings. Generally, the number of interspecific neighbors had no considerable effect on biomass or total C or N contents, as well as N partitioning in leaves and roots of beech and fir seedlings. The present study highlights that drought hardening induces memory effects in European beech and silver fir seedlings in their mixture in the subsequent year of growth, but these memory effects are stronger in beech than in fir.

Keywords: amino acids; beech; biomass; drought hardening; drought resilience; fir; growth; memory effects; mixed forests; nitrogen metabolism; roots

1. Introduction

Drought, as an environmental constrain intensified by global climate change in addition to atmospheric warming and changing precipitation, is already proven to be enhanced by global climate change in frequency, duration, and intensity, and is projected to be further enhanced in future [1]. In central Europe this scenario was seen during this century as extended summer droughts in 2003 [2] and 2018 [3] with severe injury in forestry amounting to 30% reduction in gross primary productivity [2] and unprecedented drought-induced tree mortality in many species [3] throughout Europe.

Drought impacts the morphological and physiological traits of forest trees [4,5]. The initial consequence of drought on vegetation is increased water loss from the leaves by enhanced transpiration exceeding water uptake by the roots and mediating under pressure in the xylem, causing reduced stomatal conductance, photosynthesis, leaf water potential, and sap movement [4,6–8]. Water stress can be compensated under mild drought conditions by stomatal closure [6], but severe drought results in the loss of stomatal control, leaf wilting, and leaf shedding [9]. However, stomatal closure in an O₂-containing atmosphere requires enhanced reactive oxygen species (ROS) scavenging in leaves [6], since the light reaction of photosynthesis continues to proceed, but the NADPH produced is insufficiently consumed for CO₂ fixation [10]. As a consequence, an enhanced amount of electrons is transferred to O₂ with the production of ROS. Thus, drought requires increased activity of anti-oxidative processes in leaves to prevent ROS-mediated injury [5,6,11,12].

Plants natural in arid and semi-arid environments possess structural features that reduce water loss by the leaves and counteract the negative effects of drought. These so-called xeromorphic structures include thicker leaf and cuticle, pubescent leaves and sunken stomata, and plenty of sclerenchyma, particularly found in leaves of evergreen plants such as conifers [5,13,14]. In addition, plants in temperate environments have developed features that can prevent injurious water loss and constitute a measure of drought resilience, but these features are mostly based on physiological processes [15,16]. Particularly, these features include the accumulation of compatible solutes such as carbohydrates, e.g., sugars, sugar alcohols, and polyols [17], and amino acids, e.g., proline, arginine, aspartate, and aromatic amino acids [5,6,12,18–22]. Thus, already mild drought can interact with the nitrogen (N) metabolism of plants, which constitutes an essential component of plant growth and development [5,6,23].

It has recently been reported that mild drought-mediated stomatal closure can even result in enhanced water content of the leaves [24]. This effect may be a consequence of the delay in time between stomatal closure and changes in water uptake by the roots to counteract under-pressure in the xylem [25–27]. This effect is considered to be a process of drought hardening. In mixed cultivation of European beech and silver fir seedlings in mesocosms, it has been observed that moderate drought triggered increases of relative water contents in beech leaves and current year silver fir needles and also enhanced the $\delta^{13}\text{C}$ abundance in silver fir needles. Foliar total N contents were increased in both species upon drought and decreased after rewatering. Moderate drought also resulted in higher total carbon contents in European beech leaves [24].

Increasing number of studies have reported that “stress imprint” or drought memory might facilitate a fast and protective response to a recurrent stressful event as revealed in phenotypic and physiological plasticity [28–30]. As long-living organisms, trees may particularly use epigenetics to facilitate phenotypic and physiological modifications in response to environmental changes [31]. In the present study, plant biomass, water relations, as well as carbon and nitrogen contents and fractions, were determined in mixed cultivations of beech and fir seedlings with different numbers of interspecies neighbors in the subsequent year of drought hardening [24]. Specifically, we hypothesized that (1) previous-year drought hardening has no lagged effects on water relations, but impacts biomass accumulation of both tree species; (2) memory effects can be demonstrated in both leaves/needles and roots as altered C and N contents and N partitioning; (3) the memory effects are more obvious in beech than in fir seedlings; and (4) memory effects are independent on the number of interspecific neighbors. The objective of this study was to elucidate whether drought hardening in mixed beech/fir seedlings generates memory effects in the subsequent year of cultivation.

2. Material and Methods

2.1. Plant Materials and Experimental Design

Two-year-old European beech (*Fagus sylvatica* L.) and silver fir (*Abies alba*) seedlings were bought from a commercial tree nursery (Gustav Burger Forstbaumschulen, Harmers-

bach, Germany). The mean lengths of beech and fir seedlings at planting were 21 ± 3 cm and 31 ± 4 cm for shoot, 14 ± 1 cm and 24 ± 1 cm for root, respectively. The seedlings were planted into mesocosms (size $120 \times 75 \times 60$ cm; L \times W \times H) at 15×20 cm distance, 10 of each species per mesocosm in March 2016. Within each mesocosm, the distribution of the 20 plants was designed to match 1 to 5 other species neighbors for both beech and fir, previously described in detail ([24]; see also Figure 1). This planting design was repeated 4 times. In total, 16 mesocosms with 8 replicates in every 4 mesocosms were employed. The plants were cultivated at the Chair of Tree Physiology, University of Freiburg, Germany ($48^{\circ}50.39''$ N/ $7^{\circ}50'0.51''$ E). The substrate filled in each mesocosms was a mixture of perlite and soil material collected in the black forest close to Emmendingen, Germany ($48^{\circ}02.013''$ N/ $7^{\circ}96.888''$ E) in autumn 2015 (1:1, v:v), with a layer of 3 to 5 cm leaf litter on top of the soil in each mesocosm. The litter was collected from a mixed beech/fir forest close to the soil collection site. The volumetric soil moisture was monitored during the experiment with two soil moisture sensors EC-5 (Decagon Devices, Inc., Pullman, Washington, DC, USA) previously installed at 15 cm and 37 cm depth in the mesocosms.

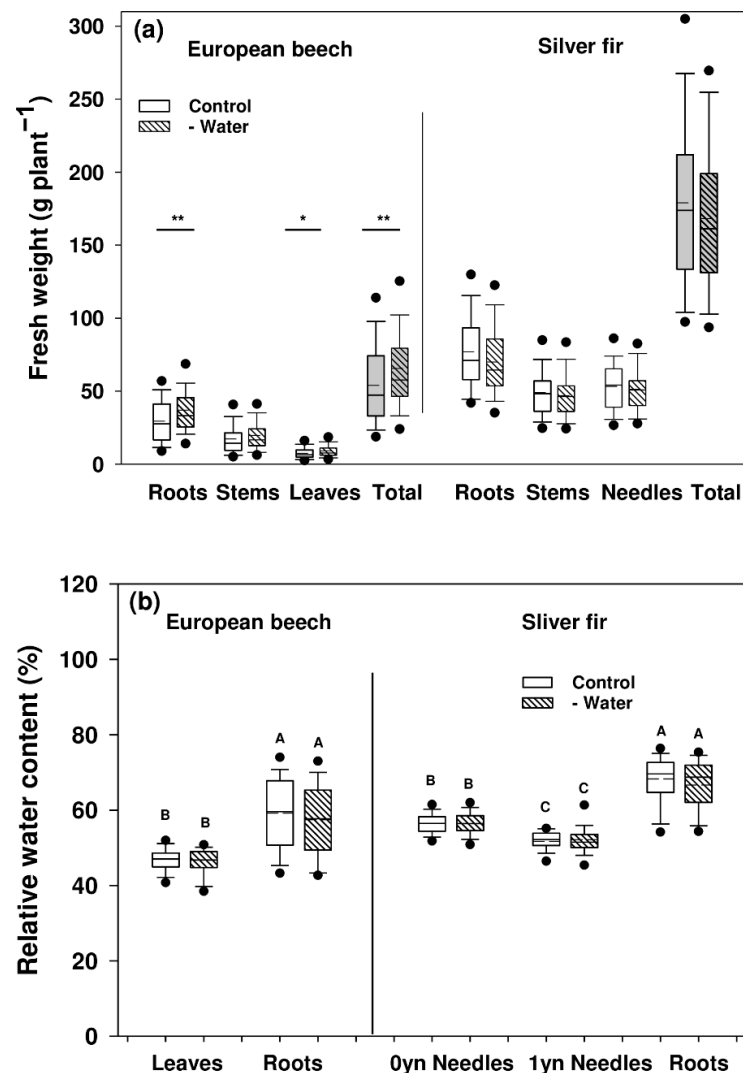


Figure 1. Fresh weight (a) and relative water content (b) of European beech and silver fir seedling after two years of growth in mixed cultivation regardless of the number of inter-species neighbors. -Water: Beech/fir mixtures exposed water deprivation and rewatering during the first year of growth. Data shown are means \pm SD (beech: $n = 75$ – 80 , fir: $n = 74$ – 80). Significant differences of fresh weight between control and -Water are indicated by asterisks (*, $p < 0.05$; **, $p < 0.01$), significant differences ($p < 0.05$) of relative water content between control and -Water are indicated by different capital letters.

2.2. Seedling Cultivation

For establishment, seedlings were well-watered and cultivated under greenhouse conditions with illumination at $150\text{--}250 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR (day/night = 16/8 h) till December 2016. Excess water was drained off through holes in the bottom of each mesocosm. Leaves of beech and fir seedlings were fully expanded after 4 weeks of plant establishment. The place of the mesocosms were randomly changed every month to minimize position effects. All mesocosms were transferred to field conditions from December 2016, and covered with UV transparent foil roofs (greenhouse film transparent, RKW AGRI GmbH, Mannheim, Germany) to prevent uncontrolled water input by precipitation, previously described in detail [24]; see also Figure S1). Thereafter, 30 L tap water per mesocosm per week was supplied according to the mean annual precipitation in Freiburg [24].

2.3. Water Deprivation and Rewatering

Water deprivation was carried out from 9 May to 27 July 2017 by withholding water [24]. Half of the 16 mesocosms were subjected to water deprivation and the remaining 8 mesocosms were used as controls and regularly watered. From 28 July to 1 August 2017, the 8 water-deprived mesocosms were rewatered gradually. For this purpose, tap water was supplied slowly until the occurrence of drain out from bottom holes, and in total 75 L tap water per mesocosm was irrigated. Subsequently, the seedlings were cultivated under identical conditions with regular watering as before until the final harvest on 2018 from 30 July to 5 August.

2.4. Sampling of Plant Material

During the final harvest, plants from control (Control) and water deprived (-Water) mesocosms of both species were carefully dug out and separated into leaves (current- and previous-year needles for silver fir), roots, and stems. Sampling took place between 09:00–15:00 to minimize diurnal variation. After determining the fresh biomass, subsamples of leaves/needles and roots were immediately immersed in liquid nitrogen. The frozen plant materials were homogenized in liquid nitrogen with mortar and pestle and then stored at $-20 \text{ }^\circ\text{C}$ fridge for biochemical analyses. Relative water content (RWC) of plant samples was estimated by $\text{RWC (\%)} = [(\text{FW} - \text{DW})/\text{FW}] \times 100$ [32]. Where FW is the fresh weight, and DW is the dry weight after drying in the oven at $60 \text{ }^\circ\text{C}$ for approximately 3 days until weight constancy.

2.5. Determination of $\delta^{13}\text{C}$ and Total C and N Contents in Plant Materials

Total N and total C contents as well as $\delta^{13}\text{C}$ signatures of oven-dried leaf and root samples were determined as previously described [24]. Briefly, 1.0–1.5 mg powder was weighed into tin capsules and measured with an isotope ratio mass spectrometer Delta V Plus (Thermo Finnigan MAT, GmbH, Bremen, Germany) coupled via a ConFlo III interface to an elemental analyser of NA2500 (CE Instruments, Milan, Italy). Glutamic acid (Sigma, Taufkirchen, Germany) subjected to the same manner was used as calibration standard.

2.6. Soluble Protein, Total Amino Acid and Structural N Determination

Soluble protein and total amino acid in plant materials were extracted and determined with photometric methods [33]. For the calculation of soluble protein and total amino acid contents, bovine serum albumin and glutamine were used as calibration standards, respectively. Structural N content was calculated as the difference between total N and the sum of N fractions of soluble protein and total amino acids [33].

2.7. Statistical Analysis

Differences between controls and treatments within the same plant material of beech or silver fir were determined using Student's t-test for normal distributed data, and the Mann–Whitney rank sum test for data which did not match normal distribution. To examine the effects of number of neighbors, one-way ANOVA was applied, followed by

the Holm–Sidak test as a post-hoc test ($p < 0.05$, $\alpha = 0.95$) for normal distributed data; otherwise, the Kruskal–Wallis One-Way ANOVA on ranks followed by the Dunn’s method was applied. Statistical analyses and figures generation were carried out with SigmaPlot 12.0 (Systat Software, San Jose, CA, USA).

3. Results

In the present study, European beech and silver fir seedlings grown in mixed cultivation and showing drought hardening after exposure to mild drought and rewetting [24] were analyzed in the subsequent year for memory effects. Drought memory was observed for biomass accumulation, C content as well as N partitioning, but differed between the two species. Memory effects related to the number of interspecies neighbors were minute for both beech and fir seedlings.

3.1. Biomass and Water Content

In European beech, drought hardening by water deprivation and rewatering mediated enhanced biomass accumulation in the subsequent year, both on a FW and DW basis (Figure 1a and Figure S1). Enhanced biomass was attributed to leaves and roots, but not to the stems. The observed effects of drought hardening on FW accumulation in the subsequent year could not be attributed to differences in water content (Figures 1b, S2 and S3). However, water content of roots was generally higher than water content of beech leaves and fir needles (Figure 1b). Increased biomass of beech was also observed, when data were separated according to the number of neighboring firs (Figures 2a and S4). A similar effect of drought hardening on biomass accumulation in the subsequent year was not observed for silver fir, but rather a decrease in biomass at 5 neighboring beech seedlings (Figures 2b and S5).

3.2. Carbon (C) and Nitrogen (N) Contents of Leaves and Roots

In European beech, total C content was decreased by drought hardening in the subsequent year, in both leaves and roots. Such a decrease was also observed in previous-year fir needles and fir roots (Figure 3a). The number of interspecific neighbors did not impact this decrease, neither in beech nor in fir seedlings (Figures S6 and S7).

The total N content of beech leaves and roots and fir needles and roots were not changed by drought hardening in the subsequent year of growth. However, in beech roots total N content was generally less abundant compared to leaves; in silver fir it was generally higher in roots than in current and previous year needles. In addition, total N content of current-year needles was higher than total N content of previous-year needles (Figure 3b). C/N ratios in beech roots were generally higher than in beech leaves and decreased as a consequence of drought hardening during the previous year. Such a decrease was also observed for fir roots. However, the C/N ratio of fir roots was similar to current-year needles and higher than in previous-year needles (Figure 3c). The number of interspecies neighbors did not affect the total N contents of beech and fir, except for a decline in the roots of beech surrounded by five fir neighbors in the controls that was prevented by drought hardening during the previous year (Figures 4 and S8). Considerable effects of the number of interspecies neighbors on C/N ratios were observed for neither European beech nor for silver fir (Figures S9 and S10).

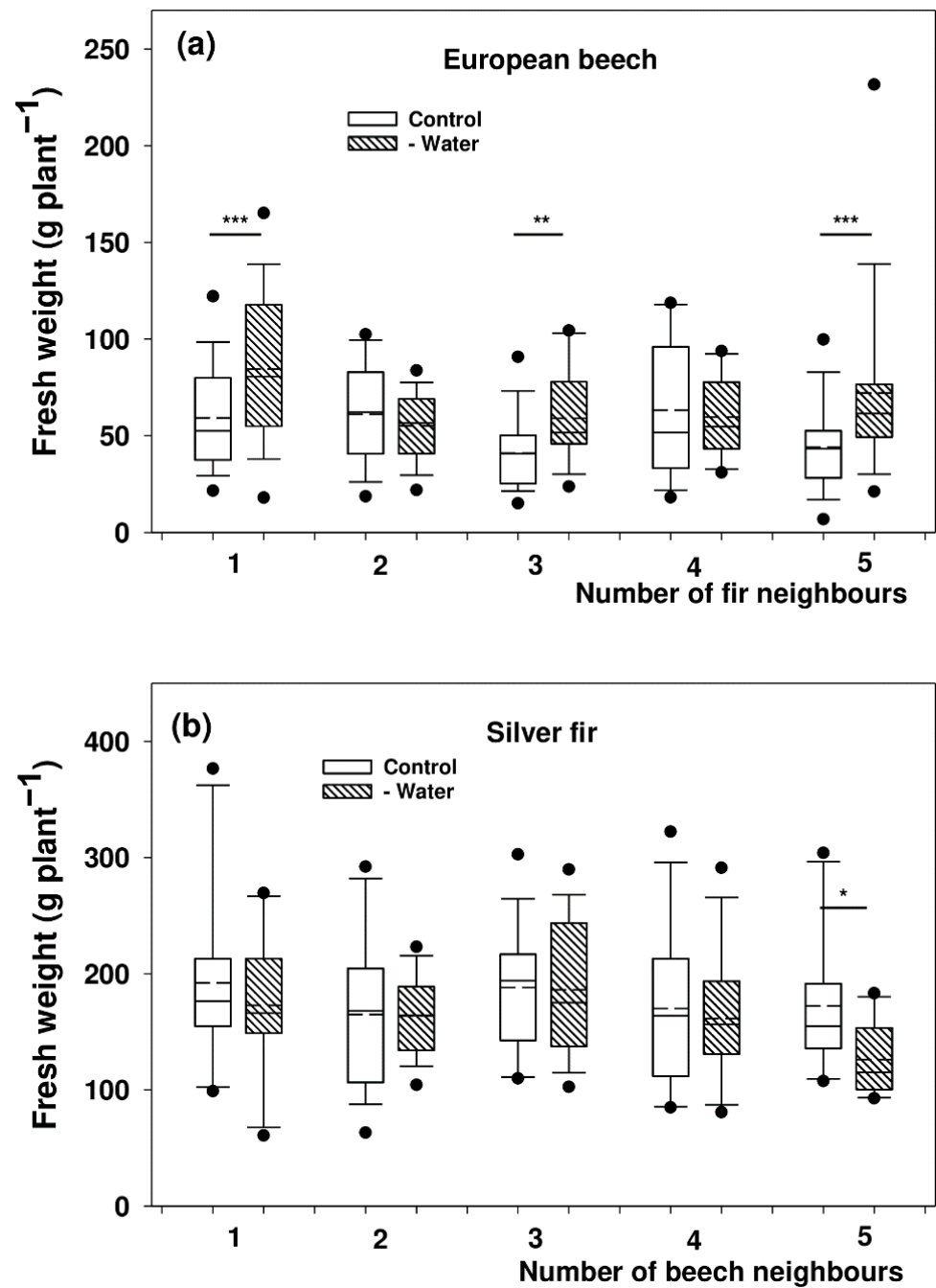


Figure 2. Fresh weight of European beech (a) and silver fir (b) after two years of growth in mixed cultivation as dependent on the number of inter-species neighbors. -Water: Beech/fir mixtures exposed water deprivation and rewatering during the first year of growth. Data shown are means \pm SD (beech: $n = 15\text{--}16$; fir: $n = 8\text{--}30$). Significant differences between control and -Water are indicated by asterisks (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

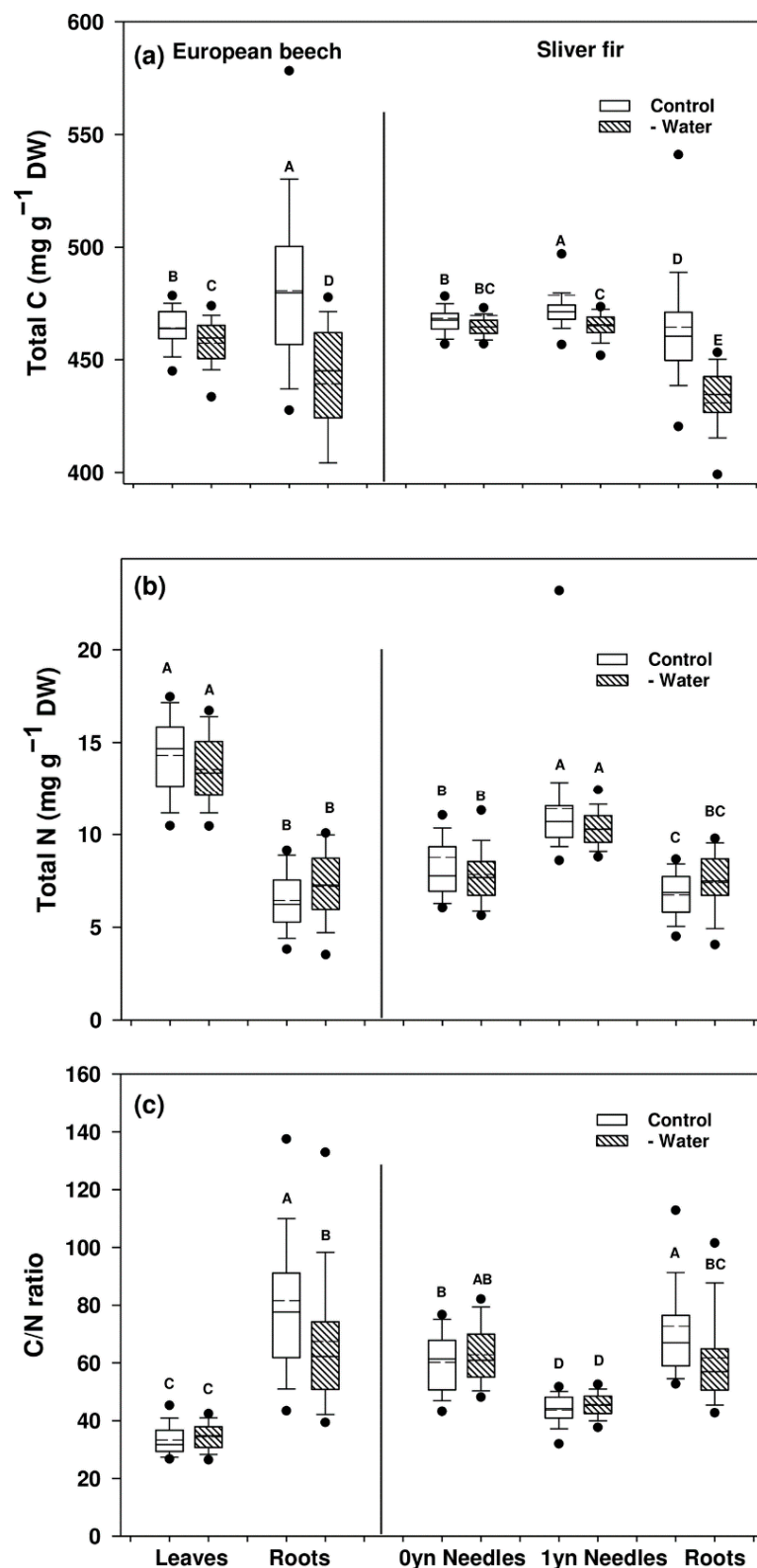


Figure 3. Total carbon (a), nitrogen (b), contents and carbon (C)/nitrogen (N) ratio (c) of European beech and silver fir seedling after two years of growth in mixed cultivation regardless of the number of inter-species neighbors. -Water: Beech/fir mixtures exposed water deprivation and rewatering during the first year of growth. Data shown are means \pm SD (beech: $n = 75\text{--}80$; fir $n = 74\text{--}80$). Significant differences ($p < 0.05$) are indicated by different capital letters.

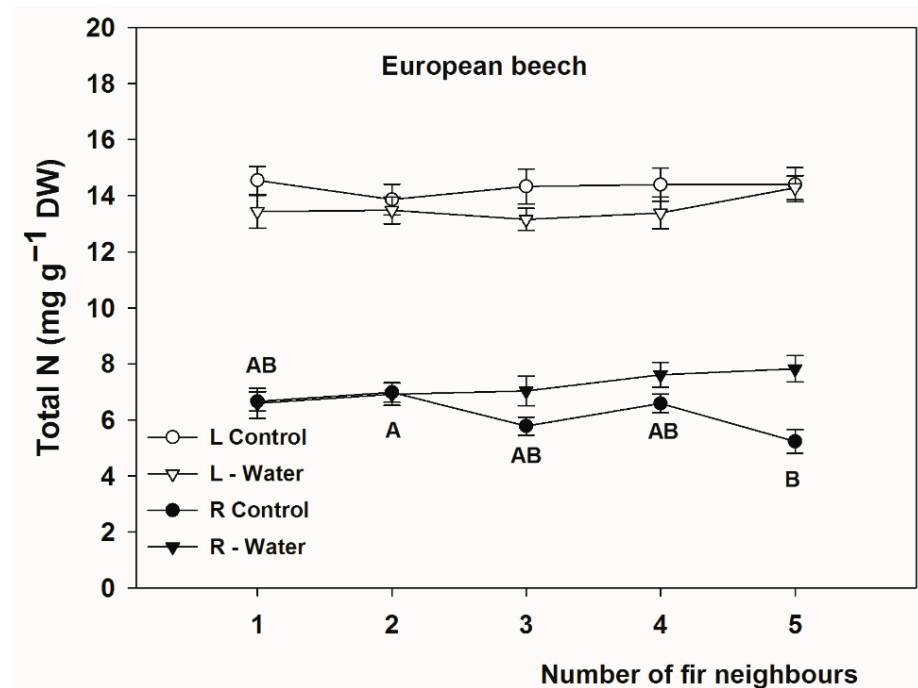


Figure 4. Total nitrogen (N) content of European beech after two years of growth in mixed cultivation with silver fir as dependent on the number of fir neighbours. -Water: Beech/fir mixtures exposed water deprivation and rewatering during the first year of growth. L: leaves, R: roots. Data shown are means \pm SD ($n = 15\text{--}16$). Significant differences ($p < 0.05$) between the number of neighbors are indicated by different capital letters.

3.3. Nitrogen Partitioning in Leaves and Roots

Total amino acid contents of beech leaves and fir needles were decreased by drought hardening in the subsequent year, whereas total amino acid contents of the roots were not affected (Figure 5a). In both species, root total amino acid contents were higher compared to beech leaves or fir needles. Differences between current-year and previous-year fir needles were not observed (Figure 5a). The number of interspecific neighbors had no effects on the total amino acid content, in neither European beech nor silver fir (Figures S11 and S12).

Soluble protein contents of beech leaves and roots or fir needles and roots were not affected by drought hardening during the previous year. However, in roots of both species, soluble protein contents were generally lower compared to leaves or needles. Differences between current and previous year fir needles were not observed (Figure 5b). Additionally, the number of interspecies neighbors did not affect total soluble protein contents in both European beech and silver fir (Figures S13 and S14).

Structural N of beech was much higher in leaves than in roots, but was not affected by drought hardening in the previous year in both organs. In silver fir, drought hardening resulted in significantly reduced structural N in current-year needles, whereas structural N in previous-year needles and roots were not impacted. Structural N content was significantly higher in previous-year needles compared to current-year needles and roots (Figure 5c). In both European beech and silver fir, the number of interspecific neighbors did not affect structural N contents (Figures S15 and S16).

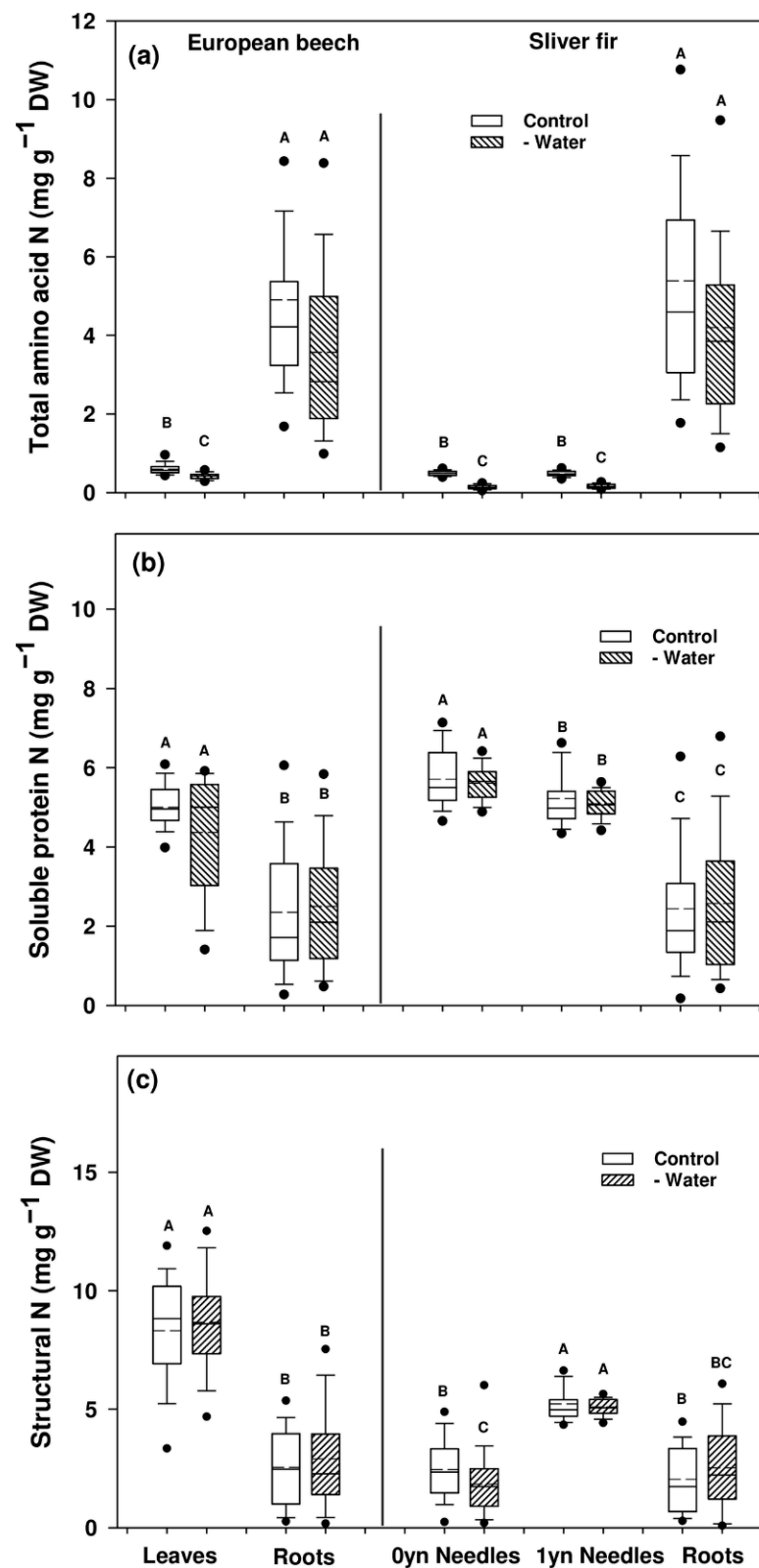


Figure 5. Total amino acid N (a), protein N (b), and structural N (c) of European beech and silver fir seedling after two years of growth in mixed cultivation regardless of the number of inter-species neighbors. -Water: Beech/fir mixtures exposed water deprivation and rewatering during the first year of growth. Data shown are means \pm SD (beech: $n = 70$ – 77 ; fir $n = 67$ – 77). Significant differences ($p < 0.05$) are indicated by different capital letters.

4. Discussion

In the context of the increasing frequency and severity of drought events as an experienced and projected consequence of global climate change [1], there is increasing interest in better understanding the mechanisms involved in plant responses to drought and to discover epigenetic changes triggering drought stress memory [5,34,35]. Plenty of studies have reported memorized information provoked by previous stress through phenotypic, physiological, and molecular changes [34,36–40]. The memorized information can modulate responses to recurring stress events, and thereby, can reduce stress susceptibility [31,35,36,41,42]. Compared to herbaceous model plants and crops, our knowledge of stress memory by forest tree species is particularly scarce [43–45]. Therefore, in the present study with beech and fir seedlings, the consequences of drought on growth, C and N contents, as well as N partitioning were documented in the year subsequent to water deprivation and rewetting.

4.1. Post-Drought Growth Differed between Beech and Fir Seedlings

Memory effects of drought generally appear as prolonged periods of growth and productivity decline [46,47]. Thus, post-drought recovery, i.e., the ability to restore vitality and growth, is an important component of drought tolerance of trees [48] and essential to predict shifts in forests dynamics and productivity in a changing climate [49]. Drought-induced delayed reductions of root and aboveground biomass as well as decreased leaf number and radial growth of beech seedlings have been reported in several studies, either in monocultures [50–52] or in mixed cultivation [53,54]. Similar effects of drought were also observed in mature beech trees [47,55–57]. Petrik et al. [58] recently reported stronger drought memory effects in beech trees on stomatal and leaf morphological traits in the subsequent year than in the year of drought exposure. This result is consistent with the present result, showing that drought altered carbon contents, observed directly after mild water deprivation and rewetting as mechanism of drought hardening [24], were largely reversed in the subsequent year (Figure 3a). Moreover, in contrast to the generally decreased root biomass observed in pure beech cultures upon drought [50–53,59,60], the facilitation of fir on beech foliar water relations and C and N status observed in the previous year [24] was also observed in the subsequent year of cultivation (Figures 1–3 and S1). The enhanced root biomass upon drought may be triggered by enhanced carbohydrate allocation to the roots [53,61–63]. Therefore, we speculate that mixed beech–fir stands can improve water availability to beech upon drought by both enhanced root biomass mediated by drought priming [34,64] and hydraulic redistribution from fir to beech [65].

Increased leaf or root biomass upon drought hardening were not observed in silver fir seedlings [24] indicating a species-specific response as also demonstrated in mature fir trees [47]. Consistent with the present results, faster recovery and less impacted gross primary productivity of beech from an inter-specific environment than in intra-specific conditions were also observed in other studies [54,57]. These results confirm the general view that angiosperms have a more rapid growth recovery upon re-watering than gymnosperms due to greater carbon reserves and higher maximum hydraulic conductivity [49,66]. Therefore, our first hypothesis has to be amended since previous-year drought hardening only impacted biomass accumulation of beech, but not of fir seedlings.

4.2. Memory Effects on Carbon and Nitrogen Metabolism

In addition to the morphological effects mentioned above, physiological changes initiated during drought stress can also be remembered by plants [31,43] as well as by soil microbial communities, which eventually may also be translated into plants traits [67]. $\delta^{13}\text{C}$ is an important integrative proxy of plant water use efficiency, water relations, and stomatal movement, and is widely employed for interpreting chemical, physical, and metabolic processes involved in carbon transformation in stressed plants [68–70]. In the present study, memory effects of drought on foliar $\delta^{13}\text{C}$ were not observed, neither in beech leaves nor in fir needles, except for a minor variation in beech roots (Figures S17 and S18). Given the

crucial role of C economy for tree growth, survival, and recovery from stress events [71–74], there is increasing interest in elaborating the memory effects of drought on the C status [73]. Previous studies indicated increased carbohydrate storage and preferential allocation of C to stem- and root- than shoot-growth in seedlings in response to drought [75,76]. On the contrary, in the present study, significantly decreased total C contents in leaves and roots of both beech and fir seedlings that experienced drought hardening were observed in the subsequent year (Figure 3a). In beech this decline in C contents may be explained by the observed stimulated compensatory post-drought growth [28,77,78], previously discussed as a strategy of stress defense rather than delayed C export from the leaves and impaired phloem transport of sugar [4,23,30,32,79]. The latter was observed in mature beech forests at repeated summer drought [80] and also demonstrated in downy oak (*Quercus pubescens* Willd.) after drought relief [81]. The decreased C contents observed in the present study are most likely due to lower non-structural carbohydrates concentrations, e.g., sucrose and fructose, previously observed in drought primed *Dipteryx alata* seedlings [38]. In addition, the lower C/N ratios of the roots of beech induced by the decreased root C contents may translate into faster fine root turnover upon recurrent drought cycles [50,82,83].

C and N metabolism closely interact with each other in plant growth, productivity, and stress resistance [84,85]. Compared to C dynamics, information on post-stress responses of N contents and partitioning in trees is extremely scarce, despite this knowledge being of particular importance for predicting the resilience of forests under climate change conditions [31,86]. The total N contents observed in this study were in the range previously found in European beech and fir [33,53,87–89]. They were largely conserved in the year subsequent to water deprivation in beech, due to stable protein contents, which is the main cytoplasmic N pool in this species [18,33,88,89]. Therefore, our second hypothesis, that memory effects are established in both leaves and roots as altered C and N contents, is only supported for C, but not for N.

Several N-containing amino acids are involved in stress response processes, e.g., osmotic adjustment, modulation of stomatal opening, ROS scavenging, synthesis of secondary defense metabolites, and signaling molecules [5,6,12,84,90,91]. Enrichment analysis showed that the biosynthesis of amino acids is involved in chloroplast-related memory transcripts in rice (*Oryza sativa* L) [37]. Accumulation of N in the form of amino acids are frequently observed in beech seedlings [24,32,92] and in mature beech-fir mixtures [33,88,89] in response to reduced water availability, as well as in other plant species under stress [5,12,21,22,91]. In the present study, strong drought-mediated memory effects on foliar amino acid metabolism were revealed by significantly declined foliar total amino acid contents of both beech and fir seedlings, whereas no significant memory effects on amino acid contents were observed in roots (Figure 4). Similarly, stronger drought effects on leaf than on root amino acid contents were reported in previous studies with beech seedlings [18], but not found for mature trees [33,88]. The decreased total amino acids contents in leaves and needles of beech and fir observed in the present study cannot be attributed to enhanced growth, since total N contents were not affected by previous year drought (Figure 3b).

The current results are consistent with a previous study showing that the most abundant proteinogenic amino compounds in beech leaves, including asparagine, glutamate, glutamine, and arginine decline upon water deprivation [18]. Similarly, memory effects of amino acid were also documented in *D. alata* leaves upon drought, with a strong decrease particularly of β -alanine [38], which is an abundant non-proteinogenic amino acid also found in beech leaves and roots [18]. The authors speculated that the decline in β -alanine was due to its degradation to donate the amino group and to form pyruvate for metabolic re-arrangements [93]. The previously observed higher soluble protein contents in leaves of mature beech and fir trees at a drier field site [88] were not observed in the current study with beech seedlings. The lower structural N in current year fir needles of seedlings that experienced water deprivation in the previous year probably indicates that more N is required for metabolic processes under these conditions, as also documented in recovering drought-heat exposed Scots pine (*Pinus sylvestris*) seedlings [86]. Although similar memory

effects on total C and total amino acid contents were observed in beech and fir, the dramatic biomass accumulations observed in beech leaves and roots were not demonstrated in fir. Therefore, our third hypothesis that memory effects are more obvious in beech than in fir seedlings is confirmed.

Previous studies showed for both beech and fir that neighborhood density impacted growth, recovery and resilience [94–96]. However, as observed for the short-term effects on C and N determined directly after mild drought and rewetting [24], a clear correlation between memory effects and the number of interspecific neighbors was not found in the current study. Therefore, the fourth hypothesis that memory effects are independent on the number of interspecific neighbors is fully supported. Little interaction of interspecific neighborhood density on growth as well as physiological drought memory is probably due to overweighed complimentary effects rather than competition, as observed in previous studies [24,33,88,97].

5. Conclusions

A good understanding of the stress memory of forest trees is an important aspect for afforestation and management, particularly in the context of the projected increasing frequency and magnitude of drought events. In the present study, with beech-fir mixtures, we found for the first time that previous-year drought hardening had strong memory effects on the biomass accumulation of beech seedlings, although water relations were not impacted. The increased beech biomass was probably due to the facilitation of fir on beech. The present study also suggests strong epigenetic memory effects on foliar amino acid contents in concert with carbohydrate metabolism. These memory effects are generally independent of the number of interspecific neighbors. The memory effects obtained from previous water deprivation are likely to alter the responses to recurrent stresses thereby improving stress resilience and post-drought growth. These findings broaden the current understanding of drought resistance of beech-fir mixtures, and will help in the afforestation and management programs. Meanwhile, the memory effect and its related drought resistance has to be further tested in detail, given the species-specific responses of beech and fir as well as the differences between seedlings and mature trees.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f13101704/s1>, Figure S1: Dry weight of European beech and silver fir seedling after two years of growth in mixed cultivation independent of the number of inter-species neighbours; Figure S2: Relative water content of European beech after two years of growth in mixed cultivation with silver fir as dependent on the number of fir neighbours; Figure S3: Relative water content of silver fir after two years of growth in mixed cultivation with European beech as dependent on the number of beech neighbours; Figure S4: Dry weight of European beech after two years of growth in mixed cultivation with silver fir as dependent on the number of fir neighbours; Figure S5: Dry weight of silver fir after two years of growth in mixed cultivation with European beech as dependent on the number of beech neighbours; Figure S6: Total carbon (C) content of European beech after two years of growth in mixed cultivation with silver fir as dependent on the number of fir neighbours; Figure S7: Total carbon (C) content of silver fir after two years of growth in mixed cultivation with European beech as dependent on the number of beech neighbours; Figure S8: Total nitrogen (N) content of silver fir after two years of growth in mixed cultivation with European beech as dependent on the number of beech neighbours; Figure S9: C/N ratio of European beech after two years of growth in mixed cultivation with silver fir as dependent on the number of fir neighbours; Figure S10: C/N ratio of silver fir after two years of growth in mixed cultivation with European beech as dependent on the number of beech neighbours; Figure S11: Total amino acid N of European beech after two years of growth in mixed cultivation with silver fir as dependent on the number of fir neighbours; Figure S12: Total amino acid N of silver fir after two years of growth in mixed cultivation with European beech as dependent on the number of beech neighbours; Figure S13: Soluble protein N of European beech after two years of growth in mixed cultivation with silver fir as dependent on the number of fir neighbours; Figure S14: Soluble protein N of silver fir after two years of growth in mixed cultivation with European beech as dependent on the number of beech neighbours; Figure S15:

Structural N of European beech after two years of growth in mixed cultivation with silver fir as dependent on the number of fir neighbours; Figure S16: Structural N of silver fir after two years of growth in mixed cultivation with European beech as dependent on the number of beech neighbours; Figure S17: $\delta^{13}\text{C}$ signatures in leaves (F) and roots (R) of European beech after two years of growth in mixed cultivation with silver fir (left panel) as dependent on the number of fir neighbours (right panel); Figure S18: $\delta^{13}\text{C}$ signatures in current year (0yn, CN) and previous year (1yn, PN) needles and roots (a) of silver fir after two years of growth in mixed cultivation with European beech as dependent on the number of beech neighbours (b). -Water: Beech/fir mixtures exposed water deprivation and rewatering during the first year of growth. Data shown are means \pm SD ($n = 74\text{--}80$). Significant differences are shown by different letters ($p < 0.05$).

Author Contributions: Conceptualization, F.Y. and H.R.; T.B. organized the plant and soil materials. Investigation, S.D., M.D. and X.Q.; Material preparation, data collection, and analysis were performed by F.Y. and D.M. The first draft of the manuscript was written by F.Y., B.D. and H.R.; Review and editing, B.D. and H.R. All authors have read and agreed to the published version of the manuscript.

Funding: The present study is part of the project “Buchen-Tannen-Mischwälder zur Anpassung von Wirtschaftswäldern an Extremereignisse des Klimawandels (BuTaKli)” within the program “Waldklimafonds” (No. 22WC106901) which was financially supported via the Fachagentur Nachwachsende Rohstoffe (FNR), Germany, by the Bundesministerium für Ernährung und Landwirtschaft (BMEL) and the Bundesministerium für Umwelt, Naturschutz, Bau und Reaktorsicherheit (BMUB) based on the decision of the German Federal Parliament.

Acknowledgments: We acknowledge support by the Open Access Publication Fund of the University of Freiburg.

Conflicts of Interest: The authors declare no conflict of interest.

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